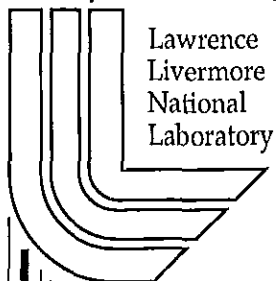


An Automated Approach for the Identification of Functionally-Relevant Small Molecule Inhibitors

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An automated approach for the identification of functionally-relevant small molecule inhibitors

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Purpose: Radiation induces the formation of DNA damages via direct ionization or through the production of reactive oxygen intermediates that chemically modify DNA. Radiation is thought to elicit its cytotoxicity by inducing the formation of lethal DNA damage, including modified bases, baseless sites and strand breaks. To avert the deleterious effects of radiation and chromosomal modifications, cells are equipped with DNA repair systems and cellular responses that function to amend genetic imperfections and to prevent the replication of damaged DNA. The focus of this proposal is to develop a novel, function-based technology for isolating inhibitors of proteins involved in radiation-protection. Such inhibitor molecules represent potential radiosensitizing agents, which could be used to increase the biological effectiveness of a given radiation dose in anti-cancer treatment schemes.

Approach: This project combines unique laboratory expertise in robotics, computational modeling, combinatorial chemistry, and DNA repair enzymology from the Biology & Biotechnology Research Program and the Chemistry and Material Science Directorate. The screening technique will utilize a simple flow-based filter system operated by robotics. Commercial laboratory instrumentation and automation are available for creating a nearly hands-off system for inhibitor molecule screening. Specifically, a general purpose dispensing instrument (i.e. the Packard Multiprobe II), using opaque, filter-backed microtiter plates, will be combined with on-deck vacuum extraction to generate a rapid screening technology. System integration tools and experience from the LLNL Human Genome Project will be leveraged. This screening capability will be applied to current lab research on proteins involved in the repair of radiation damaged DNA. Inhibitors of proteins involved in cellular resistance to radiation have potential value as co-therapeutics in anti-cancer treatments and would be licensed to pharmaceutical companies for further testing. The developed technology can also potentially be used to determine the functions of new proteins identified during the Human Genome Project. An invention disclosure has been filed for the base technology to be designed.

Technical Accomplishments: Two major repair pathways, Base Excision Repair (BER) and Recombinational Repair (RR), exist to avert the deleterious effects of radiation-induced DNA damage. In the development of an automated system, we will identify inhibitor molecules for two proteins that participate in such pathways, namely the Ape1 protein (the major human abasic endonuclease of BER) and Fen1 (a structure-specific endonuclease of RR). Since our experimentation revolves around isolating molecules

that inhibit biochemical function (i.e. molecules that prevent DNA binding, polymerization or nuclease activity), we will in essence be identifying functionally-relevant inhibitors.

In collaboration with the computational biochemistry group in BBRP, we have identified potential inhibitor molecules for Ape1 using a "docking" approach (in which candidate ligands are examined for their fit into surface cavities of a known protein structure). These and related approaches provide a valuable pre-screen for experimental assays. A similar approach is underway for Fen1. Potential inhibitors (which can be purchased or synthesized) identified by such docking methods will serve as the initial targets in our search for effective inhibitor compounds. Inhibitor screens are currently being performed for Ape1. Computational approaches will also be used in follow-up studies to determine the binding sites of the chemical inhibitors, to develop more potent inhibitors, and to draw predictive conclusions about the structure-function mechanism of the target protein. These studies are in the initial stages of development.